REVSYS: A Holistic Approach to a Holarctic Group: Subgeneric Relationships Within the Genus *Andrena* Fabricius (Hymenoptera: Andrenidae) with a Revision of the Subgenus *Callandrena* Cockerell

Project Summary

The bee genus *Andrena* is extremely large (1400+ species), and its evolutionary relationships, or phylogeny, are only poorly understood. The species are distributed throughout the northern hemisphere (a Holarctic distribution), but species groups have thus far only been treated regionally (e.g. New World species, European species, Asian species), and treatments have all been based on morphology. The morphology of *Andrena* is misleading because it is fairly uniform and prone to convergent evolution. For example, preliminary data shows that the subgenus *Callandrena* actually comprises two or more distantly-related groups that independently evolved branched pollen-carrying hairs and shortened mouthparts; other subgenera likewise do not share common ancestors.

This project will result in a molecular phylogeny, based on DNA sequences, for about 550 species of *Andrena*. International collaborators from Europe, Japan, Mexico and the United States have agreed to provide either DNA sequences or bee samples for sequencing by the Principal Investigator, Dr. Leah Larkin. The P.I. will work with a statistician and a computer scientist at the University of New Mexico on new approaches to data analysis; she will compare several new methods for their speed and accuracy.

Guided by the molecular phylogeny, the P.I. and collaborators will revise the subgenera within their respective geographic regions, including the Americas, Europe and Asia; Holarctic groups will be treated together. The P.I. will be responsible for the American groups and will focus on the subgenus *Callandrena* which, as currently delimited, is not descended from a common ancestor. She will identify diagnostic morphological characters using the phylogeny and appropriately place the species being removed from the subgenus. The "true" *Callandrena* is a group centered in Mexico with 40 or more undescribed species, which will be described. Electronic, interactive keys to *Callandrena* species and to the nearctic subgenera will be made available. A German collaborator will produce a key to the subgenera worldwide which will be adapted by the P.I. to an interactive, electronic format.

The **intellectual merit** of this study lies in the generation of a global phylogeny of what may be the largest genus of bees and in the comparison of new methods of analysis. The interactive keys will allow easy identification of species in North America. The phylogeny will aid the future study of interesting ecological interactions of the bees, including their pollen host-plant preferences, parasite relationships, and historical biogeography.

This project has **broader impacts** to the scientific community in that it will improve museum collections of *Andrena;* enhance the tenure prospects of a female P.I.; foster collaborations across disciplines and across continents; and train undergraduate students in the fields of taxonomy and systematics. Because the University of New Mexico is designated a Minority-Serving Institution, undergraduate involvement will likely increase minority participation in science.

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I. Introduction

The 20,000 or more species of bees are the most important insect pollinators of agricultural crops; they are vital to the out-crossing success of many native species, as well (Michener, 2000). Despite the crucial role that native bees play in plant reproduction, most phylogenetic work has focused on honey bees and bumble bees. Honey bees, however, are of limited effectiveness in pollinating many native plants (Westerkamp, 1991; Batra, 1995), and their populations are declining due to a number of factors, including range expansion of the Africanized honey bee and parasitic infestations by *Varroa* and tracheal mites. Thus, the role of native bees in agriculture and in native ecosystems becomes even more essential. Our limited understanding of the evolution of such ecologically important organisms constrains our ability to identify and cultivate native pollinators of plants of interest, to predict species-specific interactions in nature, and to understand the evolution of bee-parasite interactions.

The genus *Andrena* Fabricius (Hymenoptera: Andrenidae) may be the largest genus of bees, with more than 1400 described species (Michener, 2000; Gusenleitner and Schwarz, 2002) distributed across temperate regions of the northern hemisphere (a Holarctic distribution), with a few species occurring in the tropics of Central America, Africa and Southeast Asia. The recently published *Worldwide Checklist of the Bee Genus Andrena* (Gusenleitner and Schwarz, 2002) recognizes 515 species in North and Central America and 931 in Europe, Africa, India and Asia. Because so few species occur in the tropics, in this proposal these distributions will be called Nearctic and Palearctic, respectively, for simplicity. Three species are Holarctic in distribution.

Andrena females are characterized by large facial foveae, which are impressions covered with velvety hairs interior to the compound eyes; pollen-collecting hairs on the hind legs known as the trochanteral flocculus and the tibial scopa; and hind basitarsi which are at least half as long as the tibiae. Males are best identified by genital characters. The morphological uniformity of the genus has forestalled its being split into smaller genera, despite its unwieldy size and Holarctic distribution. Indeed, the 95 or more currently recognized subgenera are poorly delimited. In many cases, they are based on suites of morphological characters, some of which are shared by members of other subgenera, rather than on unique synapomorphies. Many such characters are adaptations to collecting pollen from different hosts. These are expected to be evolutionarily labile and subject to convergent evolution.

The evolutionary relationships among *Andrena* subgenera remain poorly understood. A recent molecular phylogenetic analysis (Larkin, 2002; Larkin *et al.*, submitted) has shown conclusively that the *Andrena* subgenus *Callandrena* Cockerell is not monophyletic and, despite limited sampling outside *Callandrena*, that several other subgenera are likewise not

monophyletic (see below). Finally, even the composition of *Andrena* is subject to revision; recent molecular and morphological data support the placement of the morphologically divergent *Melittoides* Friese, considered a distinct genus by some (Michener, 2000), as a subgenus within *Andrena* (Ascher, 2003).

Clearly, a delineation of the natural groups and the preparation of a classification scheme predictive of evolutionary relationships must be based on a revised suite of morphological characters. These characters should be identified based on a phylogeny derived from independent data such as DNA sequences; sequence data are less subject to the convergent selection that has complicated taxonomy of *Andrena* to date.

In addition to clarifying the relationships within *Andrena*, a robust phylogeny of the genus will allow the study of the evolution of several fascinating ecological traits. For example, many species of bee specialize on pollen-host plants while others are rather generalist, yet little is known about the evolution of this specificity. *Andrena* includes both generalists and specialists, and the Principal Investigator has shown that, among the species sampled, specialization appears to be the ancestral trait, while generalist behavior has evolved several times (Larkin, 2002). However, the sampling for this analysis was limited to Nearctic species and did not represent the breadth of *Andrena* subgenera. A better understanding of the phylogeny of *Andrena* will shed additional light on the evolution of diet breadth. Such knowledge may ultimately be useful in identifying possible pollinators that could be cultivated for agricultural or conservation purposes.

Andrena are also party to two host-parasite interactions of interest. They are hosts to internal *Stylops* parasites (Order Strepsiptera), the eggs or larvae of which are collected with pollen by female bees and ingested by the bee larvae. The parasites develop internally in the host and emerge between its abdominal segments. Female *Stylops* remain in the host; they consist only of an exposed cephalothorax and an egg sack. Males are free-flying but are rare in collections. The parasite alters the development and behavior of host bees, often inducing hermaphrodite morphology and causing them to shed *Stylops* eggs on flowers, thus continuing the cycle. Due to the extreme morphological reduction of *Stylops* and the consequent difficulty in identifying them to species, little is known of their host-specificity. Pekkarinen (1997) found no correlation between diet breadth and level of stylopization and concluded that *Stylops* are not strictly host-specific. However, interactions between bee and host-plant are certain to play a role in infection rates. Analogous work with phytophagous insects suggests that specialization may evolve as a strategy to avoid generalist enemies (Bernays, 1988; 1989).

Many *Andrena* species are also subject to cleptoparasitism by *Nomada* bees. *Nomada* do not collect pollen to provision their own nests. Rather, females enter the host's burrow and deposit two to four eggs in a cell provisioned by their host bee; the first *Nomada* larva to hatch devours both the pollen provision and the remaining bee eggs, including the host's. *Nomada* appear to be host specific (Broemeling and Moalif, 1988; Riddick, 1993; Neff and Simpson, 1997). An understanding of the evolutionary relationships of *Andrena* will set the

stage for future studies of the evolution of the interactions with both their *Stylops* parasites and their *Nomada* cleptoparasites.

Andrena bees are excellent models for testing vicariant versus dispersal biogeographic scenarios because they do not easily cross large bodies of water (LaBerge, 1986a). For example, *Andrena* are absent from the Caribbean isles, and the faunas of Britain, Japan, Taiwan and the Canary Islands are depauperate relative to adjacent continental regions (LaBerge, 1986a). The absence of *Andrena* in most southern hemisphere regions suggests that the genus evolved in Laurasia after its division from Gondwanaland in the mid-Cretaceous; the eight species in Africa south of the Sahara are likely recent migrants. Fossil evidence of *Andrena* from Colorado Florissant shale indicates that the genus had evolved by the Oligocene or early Miocene; fossils of less certain affinity from Baltic amber of the lower Oligocene place the origin in or before the early Tertiary (LaBerge, 1986a). Thus, *Andrena* appears to have evolved during a period of transient land connections that alternately connected eastern North America with Europe and western North America with Asia. Subgenera that have similar Holarctic distributions could potentially have quite different biogeographic histories.

II. Research Objectives

The P.I. of this proposal will:

- Coordinate an international team of *Andrena* specialists to work collaboratively to meet the goals of the project;
- Generate a phylogenetic hypothesis, based on DNA sequence data from both mitochondrial and nuclear markers and in collaboration with international colleagues, of a representative sampling of the global diversity of *Andrena* species;
- Identify, using the molecular phylogeny and in collaboration with international colleagues, morphological characters on which to base a natural classification of *Andrena* and recircumscribe the subgenera to reflect natural, monophyletic groupings;
- Revise the Nearctic subgenus *Callandrena s. str.*, including the description of the 40 or more new species known from Mesoamerica and the removal from the subgenus the distantly related species formerly ascribed to *Callandrena;*
- Produce an interactive key to the species of subgenus *Callandrena,* and, as time permits, to other Nearctic species;
- Supervise undergraduate students as full participants in the project.

III. Proposed Research

***Molecular phylogeny of* Andrena**. A rigorous phylogenetic hypothesis for the 1400+ species of *Andrena* is currently lacking. This project will fill this void by generating a robust molecular phylogeny for about 550 species of *Andrena* sampled from the genus' essentially Holarctic distribution (Table 1). *Andrena* species occur in the Nearctic south to the canal region of Panama; throughout Europe and in northern Africa, with only eight species south of the Sahara Desert; and as far south as the Malay peninsula in Asia. The

P.I. has assembled an international team of collaborators to ensure the broadest sampling possible of both *Andrena* species and relevant outgroups. Dr. Osamu Tadauchi of Kyushu University, Japan, will provide Asian specimens preserved in ethanol. Andreas Dubitsky, a German graduate student studying the *Andrena* subgenus *Micrandrena* under the supervision of Dr. Klaus Schoenitzer at Zoologische Staatssammlung Muenchen, will make available DNA sequence data of two markers for species relevant to his dissertation. Dr. Ricardo Ayala of the Instituto de Biologia, UNAM, Mexico, will sample bees from Mexico along with the P.I. and will obtain collecting permits for that country. The P.I. will be primarily responsible for collections from the United States. Dr. John Neff, of the Central Texas Melittological Institute, will supplement the taxon sampling from North America. John Ascher, who will begin a postdoctoral fellowship on higher-level andrenid phylogeny at the American Museum of Natural History this fall, will contribute additional Nearctic and European exemplars of *Andrena* as well as outgroups in the family Andrenidae, to which *Andrena* belongs. His sampling includes all six genera in the subfamily Andreninae, and all diverse lineages of the andrenid subfamily Panurginae.

Current and anticipated taxon sampling are shown in Table 1. The P.I. and collaborators currently have either DNA extractions or bee exemplars preserved in ethanol for 213 species in 45 subgenera, including 16 Nearctic, 11 Palearctic and 18 Holarctic subgenera. Dr. Tadauchi has just returned from collecting in central Asia with many recent accessions of *Andrena* yet to be identified. With ongoing collecting effort, the P.I. hopes to sample all subgenera, with particularly dense sampling in those suspected of being polyphyletic and of those with Holarctic distributions. This will allow the collaborators to better ascertain the monophyly or non-monophyly of these groups. The 213 species in the current sampling are nearly 40% of the expected total.

The molecular phylogeny will be based on DNA sequences from both the nuclear and mitochondrial genomes. Data acquired thus far include 2422 base pairs (bp) and have proven effective at resolving relationships within *Andrena* at the species and subgeneric level (Figure 1). Sequences include a 695 (aligned) bp region of mitochondrial DNA including the 3' end of cytochrome oxidase subunit I (COI), the transfer RNA for leucine (trnL) and 563 bp of cytochrome oxidase subunit II (COII; the marker is simply called COII here) and 1727 (aligned) bp of the F2 copy of nuclear elongation factor 1 (EF-1). A recent study of the number of characters required to resolve large phylogenies (Moret *et al.*, 2002) suggest that at least 4000 bp of aligned data will be required to resolve a phylogeny of 550 species. Additional mitochondrial data will be obtained from cytochrome oxidase subunit I (COI); 744 bp have already been sequenced for a sampling of sixteen taxa. This will be supplemented with nuclear data from the following single-copy genes that have been shown to provide informative variation at the species or subgeneric level in bees: opsin (1000 bp; Ascher *et al.*, 2001; J. Ascher, personal communication), wingless (450 bp; Brower and Egan, 1997) and arginine kinase or argK (600-800 bp; Kawakita *et al.*, 2003). Primers for these markers in bees are available. Other single-copy nuclear markers for bees, including DDC (Fang *et al.*, 1997) and PEPCK (Friedlander *et al.*, 1996),

are in development by Sean Brady in the lab of Bryan Danforth at Cornell University. Introns of these nuclear markers should be particularly useful for resolving species-level relationships, while coding regions, which evolve more slowly, will resolve subgeneric-level relationships.

The University of New Mexico's PCR and sequencing facilities include all the equipment necessary for the high through-put sequencing necessary to complete the molecular aspect of this project. Notable features include: MJ Research Tetrad PCR machine, which has four independently programmable 96-well blocks and a temperature gradient; an ABI 377 sequencer and a full-time technician to operate it; a gel rig and power supply for agarose electrophoresis; ultra-, micro-, and drying centrifuges; and a UV spectrophotometer for determining DNA concentrations. The Department of Biology also has a network license to the program Sequencer 4.0 (Gene Codes Corporation, 2002), which is used for editing sequence data. Funds for a computer for phylogenetic analyses are requested as part of this proposal; additional analyses can be performed by the collaborator John Ascher, who will have access to a parallel array of computers at the American Museum of Natural History.

Sequence alignment of coding regions for these taxa is trivial and requires no special considerations. However, the mitochondrial trnL, the intron regions of EF-1 and presumably the intron regions of other single-copy nuclear genes such as opsin (Kawakita *et al.*, 2003) are variable in length. Analyses will be performed with and without the length variable regions of the aligned dataset included and with more than one possible alignment. Alignments will be generated both manually and using computer programs, such as ClustalX (Thompson *et al.*, 1997), MegAlign (part of the DNAStar package), and POY (Giribet, 2001).

Currently, there is no consensus in the systematics community as to the best way to analyze data. Both parsimony and likelihood-based methods have advantages and disadvantages. A model-based approach would seem preferable given the complex nature of molecular evolution. Different genes evolve according to different rate and substitution parameters; for example, the base composition of the mitochondrion in insects is strongly A-T biased (Clary and Wolstenholme, 1985; Frati *et al.*, 1997). Model-based approaches such as maximum likelihood (ML) allow for parameterization of the data that more accurately reflects DNA evolution. The origin of Andrenidae around the Cretaceous-Tertiary boundary (LaBerge, 1986a) and its position near the base of bee phylogeny (B. Danforth and S. Sipes, personal communication) makes analyses vulnerable to long branch attraction (Huelsenbeck, 1998), to which likelihood is more robust than parsimony. Finally, likelihood analyses make testable the interesting evolutionary and ecological hypotheses (Huelsenbeck and Crandall, 1997; Goldman *et al.*, 2000) regarding host-plant usage, parasite relationships and biogeography discussed above.

However, a dataset as large as that proposed here poses problems for analysis by likelihood-based methods. The number of possible trees increases approximately exponentially with the number of taxa, and the time required to calculate likelihood scores

with complex models of evolution becomes prohibitive, so heuristic methods are mandatory. Parsimony analyses are much faster and have thus far been the only feasible method for very large datasets. Recently, several fast likelihood-based analyses have been published which might be capable of handling large datasets. These include the maximum-likelihood ratchet (Vos, 2003), Lewis' genetic algorithm (Lewis, 1998), and the stochastic search algorithm (Salter and Pearl, 2001).

Another approach is the disk-covering methods (DCM; Huson *et al.*, 1999), which use a "divide and conquer" strategy. DCM breaks the taxon sampling down into smaller, overlapping subsets for analysis, then reassembles the subtrees into a global tree using "supertree" algorithms (Bininda-Emonds *et al.*, 2002). DCM is a "meta-method", meaning that the strategy of analyzing subsets and later recombining the results is independent of the actual search strategy. Thus, any of the "fast-ML" strategies discussed above can be used in conjunction with DCM to expedite analysis. These alternate approaches to large likelihood problems have never been compared to one another for speed and for their ability to find the most likely tree using datasets containing more than about 200 taxa. *Andrena* sequence data will be analyzed by each of the fast-ML methods in turn to test their ability to handle large datasets. Analyses can be limited to a fixed amount of time, so they can be run from multiple starting points for fixed time frames and needn't run indefinitely. Whether the fast-ML methods successfully analyze the data or not, they will also be used in conjunction with DCM. Drs. Tiffani Williams, who is pursuing DCM for likelihood applications, and Laura Salter, author of the stochastic search algorithm, both of the University of New Mexico, have agreed to assist with these large-scale analyses. Analyzing the data in this manner will serve two purposes: it will increase the chances of finding the global ML tree for *Andrena*, and it will provide a comparison of different analytical methods valuable to the broader scientific community.

Results from the likelihood analyses will be compared to those of both maximum parsimony using PAUP* 4.0 and to Bayesian analysis using MrBayes 3 (Ronquist and Huelsenbeck, in press). Bayesian analysis also employs a rapid mode of searching tree space in a likelihood context, and results in statistical support values for branches, which can prove useful for assessing the credibility of monophyletic clades. However, it has some drawbacks for the purposes of this study. First, the number of "generations" required to achieve a stable optimum is uncertain. At a recent conference, Dave Swofford presented results from analyses run for "an insane number of generations" showing continued improvements in likelihood score at generations far exceeding the number used by most systematists (Evolution conference, Chico, CA, 2003). Second, Bayesian analysis yields statistical support for branches based on majority rule consensus, rather than the single most likely tree that will be necessary for the statistical tests proposed above. Finally, Bayesian analysis in its current implementation often results in much higher levels of support for nodes than the parsimony bootstrap (Wilcox *et al.*, 2002) and which may not be realistic.

Should all attempts to use likelihood fail to successfully analyze the data, parametric bootstrapping of parsimony results with the SOWH test (Goldman, *et al.*, 2000) will still allow statistically-based tests of the evolutionary hypotheses discussed above.

Biogeographic hypotheses of the origins of the clades can be investigated using the computer program r8s (Sanderson, 2002b). R8s uses a penalized likelihood modification (Sanderson, 2002a) of non-parametric rate smoothing (Sanderson, 1997) to estimate dates of nodes on a tree even when a molecular clock is not applicable. In the case of *Andrena*, reference dates can be assigned to nodes based on the fossil record and supplemented with date ranges based on tectonic events. For example, a closely-related species pair from the southern tip of Baja California (*Andrena (Callandrena) manifesta*) and from Guerrero (an as-yet undescribed species identified by the P.I.) will allow for a date of approximately 5.5 million years to be assigned to a branch on the phylogenetic tree; this is the presumed time of the opening of the Gulf of California (Grismer, 2000). Using these assigned dates, r8s can infer the ages of the nineteen subgenera with Holarctic distributions, should they prove monophyletic, potentially distinguishing their distributions as resulting from land connections between eastern North America and Europe or between western North America and Asia.

Predictive natural classification of* Andrena *subgenera. The bee genus *Andrena* contains over 1400 described species (Gusenleitner and Schwarz, 2002) distributed Holarctically. The exact number of subgenera is not established: Michener (2000), in his *magnum opus* on bees, recognized 95, while the recent checklist of *Andrena* by Gusenleitner and Schwarz (2002) recognized 98, with an additional 41 species not assigned to subgenus. While the essential monophyly of the genus is not in doubt, there is debate over the placement of *Melittoides;* Michener (2000) considered it a genus and Gusenleitner and Schwarz (2002) a subgenus of *Andrena*. Recent molecular and morphological evidence suggests *Melittoides* does in fact belong in *Andrena* (Ascher, 2003; J. Ascher, personal communication; see Figure 1). With this one exception, there is little difficulty distinguishing *Andrena* from other genera, but delimitation of the subgenera is hampered by the morphological uniformity of the species.

Andrena as a whole has never been revised, but there have been extensive revisions of some regional groups based on morphology. For example, W. E. LaBerge and colleagues (LaBerge, 1989 and references cited therein) revised nearly all the Nearctic subgenera. Hirashima, Tadauchi and colleagues (c.f. Tadauchi and Xu, 2000; 2002; Xu and Tadauchi, 2002) treated many Asian subgenera; Osychnyuk (c.f. Osychnyuk, 1984; 1993; 1994) considered many species from the former USSR; and Warncke and others (Warncke, 1967; 1968; Dylewska, 1987) took up the European groups. Despite these revisions, the monophyly of the subgenera has not been established, and phylogenetic relationships within the genus *Andrena* remain largely unresolved. A fundamental problem is the regional nature of the treatments. Of the 19 subgenera with Holarctic distributions, only two have been treated in their entirety. Subgenus *Charitandrena* Hedicke (LaBerge, 1969)

has only two species, and subgenus *Taeniandrena* Hedicke has only one species in the Nearctic; this species itself is Holarctic in distribution.

Of the remaining subgenera, 32 are distributed only in the Nearctic and 47 solely in the Palearctic regions. Neither the Holarctic nor the regional subgenera have been shown to be monophyletic. To the contrary, recent molecular phylogenetic work (Larkin, 2002; Larkin *et al.*, submitted) demonstrates conclusively that one Nearctic subgenus, *Callandrena*, is not monophyletic as currently delimited (LaBerge, 1967). Sampling of 46 of the 80 species that had been described at the time, plus eight new species, indicates that *Callandrena* comprises at least two distantly related and possibly four clades of bees. These species have in common the character states of branched scopal hairs and shortened mouthparts (among others). These morphologies are likely convergent adaptations to shared host plants in the family Asteraceae. Data from this work was analyzed with additional sequences provided by John Ascher to suggest that other subgenera, e.g. *Gonandrena* Viereck, *Melandrena* Perez, *Plastandrena* Hedicke, *Ptilandrena* Robertson, *Rhacandrena* LaBerge, *Scaphandrena* Lanham, and *Scrapteropsis* Viereck, are not natural groups either (see Figure 1). These results should be treated cautiously due to the limited sampling of these groups. Other apparently unnatural subgenera are *Micrandrena* Ashmead, *Fumandrena* Warncke, and *Ulandrena* Warncke (A. Dubitsky, personal communication).

Species relationships among subgenera as revealed by these molecular phylogenetic analyses differ from those predicted by traditional morphology-based classification schemes. LaBerge (1980) believed that the subgenera *Parandrena* Robertson, *Gonandrena,* and *Belandrena* Ribble were closely related to subgenus *Andrena s. str.*, yet these relationships are not supported by the preliminary phylogeny (Figure 1). Also, he hypothesized a relationship between subgenus *Ptilandrena* and the subgenera *Andrena s. str.* and *Cnemidandrena* Hedicke (LaBerge, 1986b) which is likewise contradicted by the tree.

In conjunction with international colleagues, the P.I. will produce a morphology-based classification scheme for *Andrena* that is predictive of evolutionary relationships. The P.I. will be responsible for incorporating species of Nearctic *Andrena* into this scheme. In conjunction with this work, the P.I. will produce a practical guide for identifying Nearctic *Andrena* that will be accessible electronically. This guide will include a traditional dichotomous key as well as an interactive key to species (see below). As morphology alone has not proven adequate to the task of resolving *Andrena* subgeneric relationships, the molecular phylogeny generated as a result of this work will be essential in identifying natural groups. It will prove a valuable complement to the dissertation work of Andreas Dubitsky which includes a phylogenetic analysis of external adult morphology of all *Andrena* subgenera, with particular focus on the western Palearctic region, while the P.I.'s work centers on the Nearctic region. Joint evaluation of morphological characters and character states in light of the phylogeny should reveal which are most appropriate to

create a natural classification. Ideally, these characters will also be predictive, in that species discovered in the future will be easily and accurately placed to subgenus.

Morphological characters of diagnostic value will be identified by coding characters for the species and subgenera sampled in the molecular phylogeny and mapping them onto the tree using parsimony in the program MacClade 4.0 (Maddison and Maddison, 2000). For the Nearctic species, this will be a relatively simple task. The species descriptions in the revisions of LaBerge and colleagues (LaBerge, 1989 and references cited therein) are quite detailed. They identify characters deemed important for species and subgeneric relationships and will serve as initial guides for characters and character states. This strategy was successfully applied to identify deep facial foveae as a diagnostic character for the subgenus *Callandrena* (Larkin, 2002), discussed below. Undergraduate researchers will be able to use these guidelines for coding of actual character states using both female and male museum specimens for each species sampled.

Revision of subgenus* Callandrena *s. str. The largest entirely Nearctic subgenus of *Andrena* is *Callandrena. Callandrena* comprises 81 currently described species in North America (four more new species descriptions are in press; Larkin, in press), ranging in distribution from southern Canada to the Republic of Panama, and from the east coast to the west in the United States. In Mexico, most species are found in the central highlands, but a few range northward into the Chihuahuan and Sonoran deserts and one is endemic to southern Baja California. The greatest diversity of species occurs in the southwestern United States and in central Mexico, where a majority of the species are endemic (LaBerge, 1986a) and most are undescribed (D. Yanega, personal communication, and the P.I.'s observations).

Like most North American bee taxa revised before 1970, subgenus *Callandrena* lacks a rigorous phylogenetic hypothesis. The best estimates of relationship stem from the extensive revisionary work of LaBerge (1967), who expanded *Callandrena* to include Lanham's (1949) concept of *Pterandrena* Robertson and described many new species, and from a morphological phenetic analysis by Molina-Pardo (1973). Although LaBerge believed *Callandrena* to be monophyletic, he conceded that he had found no synapomorphies for the group. Rather he delimited the subgenus by a suite of morphological characters which may not be present in all *Callandrena* species or which may appear singly in members of other subgenera. Emphasis has largely been on the length of the maxillary palpae and the branched scopal hairs as diagnostic characters, but both may be adaptations to pollen collection. Cruden (1972), for example, reported convergence of short, narrow, pointed galeae in unrelated California bees that specialize on the Hydrophyllaceae. Similarly, branched scopal hairs appear independently in species which specialize on the Asteraceae (Lanham, 1949).

The P.I. has previously shown that subgenus *Callandrena* as delimited by LaBerge is not monophyletic (Larkin, 2002; Larkin *et al.*, submitted). *Callandrena sensu stricto* is a clade basal to the *Andrena* sampled in the preliminary tree (Figure 1); it is most diverse in

central Mexico. Species can be distinguished morphologically from the species to be removed from *Callandrena* by the deep facial foveae, the borders of which are often perpendicular to the frons. LaBerge noted this character for some species, but did not recognize its diagnostic importance. The species to be removed from *Callandrena* may be more appropriately placed in subgenus *Chrysandrena* Hedicke, a Eurasian subgenus of 13 species, as suggested by LaBerge (1967; 1986a), or may constitute a new subgenus altogether. As additional taxon sampling and DNA sequencing further resolve the phylogenetic tree of *Andrena*, these species will be formally removed from *Callandrena* and placed appropriately. Inclusion of members of *Chrysandrena* in the taxonomic sampling for the molecular phylogeny is crucial to this end.

A revision of subgenus *Callandrena s. str.* will complete the major revisionary work on Nearctic *Andrena* initiated by LaBerge and is a major goal of this project. It will involve identifying diagnostic morphological characters for the subgenus, delineating it from the distantly related species formerly ascribed to the subgenus, and describing the 40 or more undescribed species known from museum collections, primarily from Mesoamerica. Descriptions will follow the detailed format of LaBerge and colleagues. This format was used by the P.I. in recent species descriptions (Neff and Larkin, 2002; Larkin, in press) of five new species which were collected in the past few years in the southwestern United States, including one from the heavily collected region of the Chiricahua Mountains of southeastern Arizona.

In addition to a recircumscription of subgenus *Callandrena* and descriptions of new species, the revision will include both a dichotomous key and an interactive key, which will be made available in electronic format on the internet (see below). This key will complement the more inclusive keys to the natural subgenera of Holarctic *Andrena* produced by the P.I. in conjunction with international colleagues. In deference to the distribution of the group, the key of the *Callandrena* species will be published in both English and Spanish.

Morphological work related to this revision will take place in the Arthropod Collection of the Museum of Southwestern Biology, which has undergone a revitalization in recent years. It occupies a newly-renovated space with compactorized collections. Two new and enthusiastic curators and several graduate and undergraduate students are actively engaged in research, grant-writing, collection databasing and collection expansion.

Production and electronic dissemination of interactive keys. Although LaBerge and his students produced a series of dichotomous keys to most of the described species in North America, in many specimens the required features are not visible. For example, most collectors do not routinely "pull" the mouthparts of *Andrena* specimens, thus rendering them visible, yet the most recent key to Nearctic subgenera (LaBerge, 1985) requires an assessment of the mouthparts at couplet 7 of 74 for females and 4 of 67 for males. These keys are also difficult to use without a degree of proficiency with the anatomy of these bees. The P.I. has devised a preliminary version of an interactive key to

species of subgenus *Callandrena sensu lato* which works on specimens even when they cannot be keyed in LaBerge's key because of these limitations. The preliminary key expands on characters used in LaBerge's (1967) key to species, including information from distributions and phenology. This preliminary interactive key will be expanded to include the currently undescribed species of *Callandrena s. str.* and to include maps of geographic distributions, which have already been produced. As time permits, interactive keys to the remaining Nearctic species will be produced; the extremely detailed descriptions of species by LaBerge and colleagues (LaBerge, 1989 and references cited therein) allow for relatively easy identification of character states, making this key a reasonable goal. Undergraduate students will be responsible for databasing specimen localities to generate the distribution maps for these species. Undergraduates will also test the keys to determine their ease of use. Finally, the key to subgenera to be compiled by the collaborator A. Dubitsky based on his morphological work will be formatted, with his permission, into an interactive key and disseminated on the internet as well.

Undergraduate participation. Undergraduate participation is an essential component of this project. Qualified undergraduate students will be selected based on their academic performance and enthusiasm for entomology, taxonomy and/or phylogenetic analysis. This project specifically targets undergraduates in order to lure unsuspecting students into taxonomy before they have chosen to focus on some other field. In the first year, students will database specimen loans. Database information will eventually be used to generate distribution maps of the species using GIS software (maps for *Callandrena s. l.* species have already been produced) for inclusion in interactive keys to Nearctic species. The data will be made available in its original, complete form to the loaning institutions.

Working with the specimens in this context will give the undergraduates familiarity with the morphology and distributions of *Andrena* bees. They will be taught to identify bees to genus using the excellent illustrated keys in *The Bee Genera of North and Central America* (Michener *et al.*, 1994), as well as the more specific morphology necessary to identify *Andrena* species in the keys to Nearctic *Andrena* (LaBerge, 1989 and references cited therein). Eventually, they will use their skills to test and improve the interactive keys to the *Andrena* species and subgenera.

Students will also participate in the generation of the DNA sequence data for the phylogeny. They will learn essential laboratory skills such as DNA extraction, PCR and sequencing, as well as phylogenetic analysis. As their skills develop, students will be encouraged to work independently, designing and executing small phylogenetic projects or describing new species, and to present their results at scientific conferences.

Ideally, an undergraduate will begin in the sophomore year and continue through to graduation. While turnover is expected, the duties and educational experiences are structured to take an inexperienced but enthusiastic student from knowledge of basic morphology and databasing through laboratory skills such as PCR and sequencing to data

analysis and alpha-taxonomy. Because of the large number of undescribed Nearctic species, undergraduates will have ample opportunity to describe and name one or more new species for publication. Additional papers focusing on the phylogeny or ecology of small species groups within *Andrena* are also possible.

The University of New Mexico is designated as a Minority-Serving Institution, with a high percentage of Hispanic and Native American students; undergraduate participation in this project is likely to accomplish NSF's goal of increasing minority representation in the sciences.

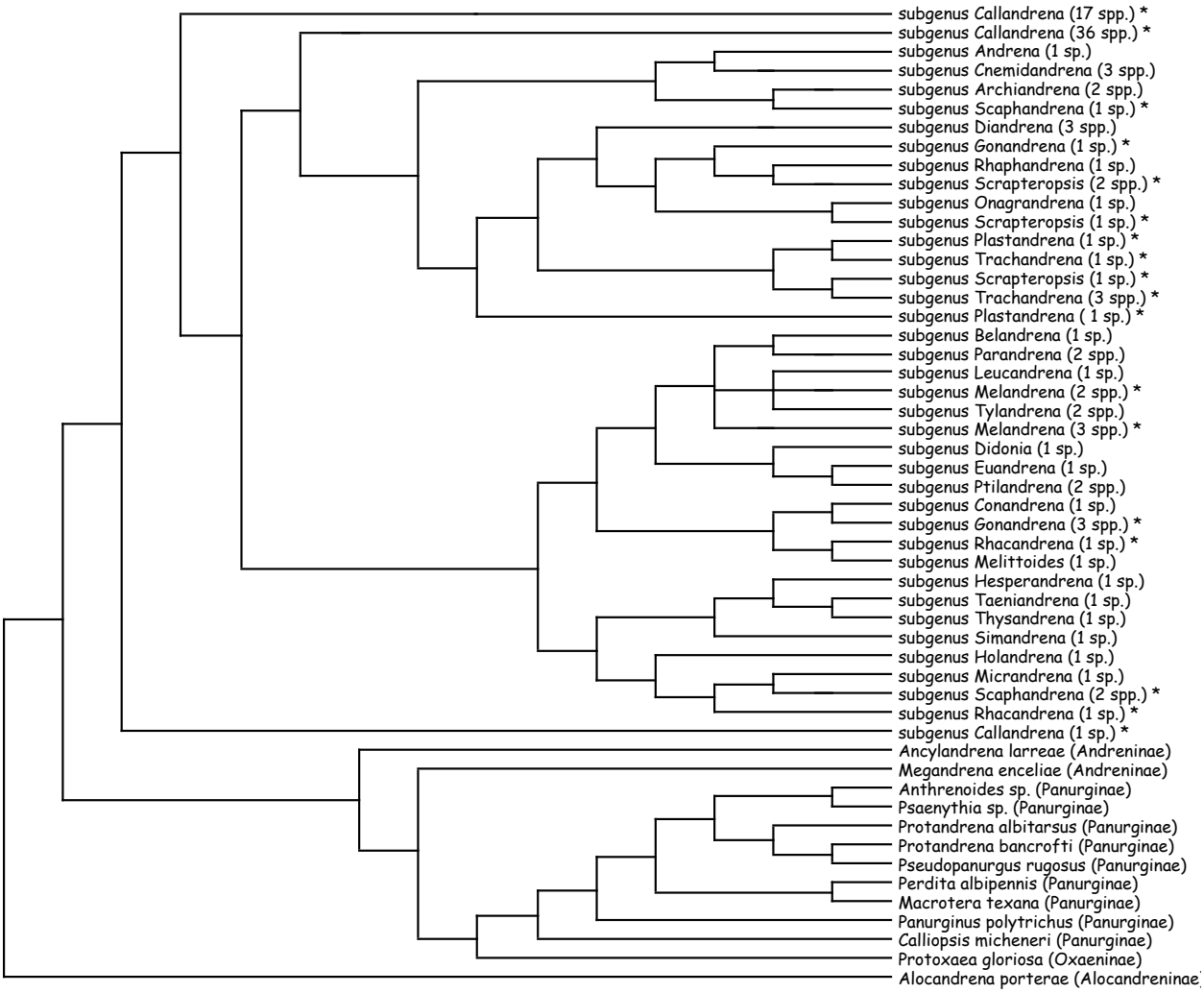
IV. Project Timeline

A. Prior to Award Commencement
- assemble primary literature of descriptions of Nearctic *Andrena* species
- continue databasing localities from the literature
- obtain specimen loans from United States and foreign museums
- begin alpha-taxonomic work on Mexican specimens of subgenus *Callandrena*

B. Year One: January - December 2004
- expand DNA sequence data sampling of *Andrena*
- expand taxonomic sampling through spring and fall collecting trips (when adults are emergent) in the United States and Mexico
- initiate morphological studies of *Andrena* subgenera
- continue alpha-taxonomy of Mexican *Callandrena*
- database specimen localities for GIS analysis

C. Year Two: January - December 2005
- continue morphological studies of *Andrena* subgenera
- expand taxonomic sampling through spring and fall collecting trips to western and southwestern United States and Mexico
- continue alpha-taxonomy of Mexican *Callandrena* and incorporate them into interactive keys
- travel to Europe to collect representatives of subgenus *Chrysandrena* and to interact with the collaborator A. Dubitsky
- complete DNA sequencing

D. Year Three: January - December 2006
- complete interactive keys to subgenera and to *Callandrena* species
- compare fast-likelihood algorithms using the *Andrena* dataset
- complete molecular phylogenetic analyses of *Andrena* species



Figures 1: Strict consensus of 16200 most parsimonious trees of length 6887, representing 107 species and 29 subgenera of *Andrena*. DNA extracts or ethanol-preserved exemplars have been collected by the P.I. and collaborators for an additional 106 species and 16 subgenera. This tree resulted from an analysis of COII and EF-1 sequence data generated by L. Larkin and EF-1 data by J. Ascher. Sequence data comprised 695 bp of COII and 1727 bp of EF-1 sequenced in two parts. Not all regions were completely sequenced for all taxa. For clarity, subgenera as currently delimited are shown rather than individual species; the number of species sampled in each clade is indicated. Asterisks mark the positions of subgenera that are not monophyletic. Of the non-monophyletic groups, species of subgenera *Callandrena* and *Scrapteropsis* each appear in three places on the tree, and species of subgenera *Gonandrena, Melandrena, Plastandrena, Rhacandrena, Scaphandrena,* and *Trachandrena* each appear in two places. Outgroups represent all four subfamilies (indicated in parentheses) of the family Andrenidae.

Table 1: Summary of current taxon sampling. Numerators indicate the number of species sampled; denominators indicate the number of described species. Asterisks denote one or more undescribed species in the sampling. Crosses mark subgenera with one holarctically distributed species; denominators do not tally for these. Sampling totals 213 species in 45 of the 98 subgenera.

subgenus	Palearctic	Nearctic	total	goal	subgenus	Palearctic	Nearctic	total	goal
Aciandrena	0/26		0/26	7	*Lepidandrena*	1/18		1/18	5
Aenandrena	0/7		0/7	3	*Leucandrena* †	1/9	2/13	3/19	8
Agandrena	1/3		1/3	2	*Longandrena*	0/3		0/3	2
Anchandrena		0/2	0/2	2	*Malayapis*	0/1		0/1	1
Andrena †	2/42	14/42	16/83	21	*Margandrena*	0/7		0/7	3
Aporandrena		0/2	0/2	2	*Melanapis*	0/1		0/1	1
Archiandrena		2/3	2/3	2	*Melandrena*	4/41	15/29	19/70	18
Augandrena		0/3	0/3	2	*Melittoides*	1/5		1/5	2
Avandrena	0/7		0/7	3	*Micrandrena*	11/86	7/21	18/107	27
Belandrena		2/5	2/5	2	*Nemandrena*		1/3	1/3	2
Biareolina	1/1		1/1	1	*Nobandrena*	0/13		0/13	4
Brachyandrena	0/4		0/4	2	*Notandrena*	1/14	0/2	1/16	7
Callandrena		55/80*	55/80*	85	*Oligandrena*		0/3	0/3	2
Calomelissa	2/6		2/6	2	*Onagrandrena*		2/26	2/26	7
Campylogaster	0/14		0/14	4	*Opandrena*	0/1	0/1	0/2	2
Carandrena	0/38		0/38	10	*Orandrena*	0/10		0/10	4
Carinandrena	0/1		0/1	1	*Oreomelissa*	0/13		0/13	4
Celetandrena		0/1	0/1	1	*Osychnyukandrena*	0/2		0/2	2
Charitandrena	1/1	0/1	1/2	2	*Oxyandrena*		0/1	0/1	1
Chaulandrena		0/1	0/1	1	*Pallandrena*	0/4		0/4	2
Chlorandrena	1/50		1/50	13	*Parandrena*	0/4	3/10	3/14	7
Chrysandrena	0/13		0/13	4	*Parandrenella*	0/8		0/8	3
Cnemidandrena	0/19	4/30*	4/49*	13	*Pelicandrena*		0/1	0/1	1
Conandrena		1/3	1/3	2	*Planiandrena*	0/4		0/4	2
Cordandrena	0/6		0/6	2	*Plastandrena*	3/27	3/6	6/33	9
Cremnandrena		0/1	0/1	1	*Poecilandrena*	3/30		3/30	8
Cryptandrena	0/5		0/5	2	*Poliandrena*	1/34		1/34	9
Cubiandrena	0/2		0/2	2	*Psammandrena*		0/2	0/2	2
Dactylandrena		1/3	1/3	2	*Ptilandrena*	0/10	3/3	3/13	7
Dasyandrena		0/3	0/3	2	*Rhacandrena*		4/4*	4/4*	4
Derandrena		3/9	3/9	3	*Rhaphandrena*		1/2	1/2	2
Diandrena		5/25*	5/25	7	*Rufandrena*	0/2		0/2	2
Didonia	1/7		1/7	3	*Scaphandrena*	0/31	2/25*	2/56	14
Distandrena	0/11		0/11	4	*Scitandrena*	0/1		0/1	1
Erandrena		0/1	0/1	1	*Scoliandrena*		0/2	0/2	2
Euandrena	3/54	5/20	8/74	19	*Scrapteropsis*		5/20	5/20	10
Fumandrena	0/11		0/11	6	*Simandrena*	3/32	3/11	6/43	11
Fuscandrena	0/1		0/1	1	*Stenomelissa*	0/3		0/3	2
Geissandrena		0/1	0/1	1	*Suandrena*	0/11		0/11	4
Genyandrena		1/2	1/2	2	*Taeniandrena* †	1/23	1/1	2/23	6
Gonandrena		5/8	5/8	4	*Tarsandrena*	0/7		0/7	3
Graecandrena	0/21		0/21	6	*Thysandrena*	0/6	2/18	2/24	8
Habromelissa	0/3		0/3	2	*Trachandrena*	0/6	8/24	8/30	8
Hesperandrena		1/7*	1/7	3	*Troandrena*	0/3		0/3	2
Holandrena	1/12	1/4	2/16	6	*Tylandrena*		3/14	3/14	4
Hoplandrena	1/24		1/24	6	*Ulandrena*	0/31		0/31	15
Hyperandrena	0/2		0/2	2	*Xiphandrena*		0/1	0/1	1
Iomelissa		0/1	0/1	1	*Zonandrena*	1/17		1/17	5
Larandrena	1/5	1/1	2/6	4	unassigned	0/15	0/17	0/32	13
Leimelissa	0/6		0/6	2	**TOTAL**	**46/931**	**167/515**	**213/1449**	**550**

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